# ANTIBACTERIAL, ANTIOXIDANT AND PHYTOCHEMICAL INVESTIGATION OF *ALBIZIA ANTHELMINTICA* LEAVES, ROOTS AND STEM BARK

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**DECLARATIONS**

I, NAWINDA TEOPOLINA N, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.



……. Date……..

**Summary**

*Albizia anthelmintica* belongs to the family *Fabaceae*. The plant is traditionally used to treat symptoms of microbial infections in both humans and animals. The present study was designed to evaluate the phytochemical content, antibacterial and antioxidant activities of *A. anthelmintica* leaves, roots and stem bark ethanol extracts. Antibacterial activity was carried out by means of the agar disc diffusion method. The anti-oxidative activities of the extracts were determined by means of ferric reducing antioxidant power ($Fe^{3+}$- $Fe^{2+}$), phosphomolybdenum reduction and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays and phytochemical screening was used to determine the major classes of compounds present in the specific plant parts. Phytochemical screening revealed the presence of alkaloids, saponins and diterpenes in roots and bark extracts, flavonoids in leaves extract, tannins in roots extracts, and phenolic compounds were detected in all three extracts. The root extract exhibited the highest total phenolic and flavonoid content of 1741.08 ± 0.05 mg GAE/100 g and 366.80 0.02 ± mg QEE/100 g, respectively. The extracts demonstrated dose dependent varying degrees of anti-oxidative efficacy in the phosphomolybdenum reduction, iron (III) reduction and 2,2-diphenyl-1-picrylhydrazyl radical scavenging assays. For the DPPH assay, $IC_{50}$ values of 0.018 ± 0.01, 0.019 ± 0.01 and 0.024 ± 0.01 mg/mL were obtained for the root, leaf and bark extracts, respectively. All extracts were active against *Staphylococcus aureus*, *Shigella sonnei* and *Serratia marcescens* but did not show activity against *Enterococcus faecalis* and *alcaligenes faecalis*. The results from the study show that *Albizia anthelmintica* extracts possess compounds with antibacterial as well as anti-oxidative properties, making this plant species a good source for potent antibacterial and anti-oxidative agents. The findings of this study will add value to the traditional uses of *A. anthelmintica*, and conservation is necessary due to the significant medicinal properties demonstrated by the plant.

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**List of Abbreviations**

| | |
|---|---|
| **AAE** | **Ascorbic Acid Equivalents** |
| **DPPH** | **2,2 – Diphenyl – 1 – Picrylhydrazyl** |
| **ET** | **Electron Transfer** |
| **FRAP** | **Ferric Reducing Antioxidant Power** |
| **GAE** | **Gallic Acid Equivalents** |
| **HAT** | **Hydrogen Atom Transfer** |
| **MIC** | **Minimum Inhibitory Concentration** |
| **MRSA** | **Methicillin Resistant *Staphylococcus aureus*** |
| **PBP2** | **Penicillin Binding Protein 2** |
| **QEE** | **Quercetin Equivalents** |
| **SPA** | **Synthetic Phenolic Antioxidant** |
| **TAC** | **Total Antioxidant Capacity** |
| **TFC** | **Total Flavonoid Content** |
| **TPC** | **Total Phenolic Content** |
| **UTI** | **Urinary Tract Infection** |
| **WIND** | **National Herbarium of Namibia** |

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**DEDICATION**

This thesis is dedicated to my late parents, my family at large. Thank you for recognising the potential in me, and believing in me at all times.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

For centuries, plants have been used as a source of medicine in many different countries and have in most instances contributed to the development of potent drugs (1). This has led to screening plants for the discovery of new bioactive compounds becoming a routine activity in many laboratories (2). According to Obiajunwa-Otteh *et al* (3), medicines derived from plants have significantly contributed towards human health. Even though there are various approaches available for the discovery of therapeutics, natural products remain the best source of novel entities (4).

Plants used for medicinal purposes have gained recognition as the richest natural source of nutraceuticals, food supplements, modern medicine, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (5). Success of herbal therapy in treating various diseases is mostly due to their safety and efficacy compared to allopathic medicine (6). The curative properties of medicinal plants are attributed to the presence of various phytochemicals (7). Phytochemicals are chemical compounds (secondary metabolites) found in plants, which plants produce for self-protection, but studies have shown that these chemicals have the ability to protect humans against various diseases (7). Some phytochemicals such as anthraquinones, tannins, terpenoids, glycosides are reportedly to have antimicrobial activities (8). In addition, flavonoids, tannins, alkaloids and phenols are reported to possess antioxidant properties (9). Medicinal plants are associated with many traditional claims, which include their use for the

treatment of ailments that are a result of infections (10). However, the traditional uses need to be scientifically validated and recorded (11).

*Abizia anthelmintica* (*Fabaceae*) is a slow growing tree that belongs to the genus *Albizia* (12). The genus *Albizia* houses around 150 species, of which most are trees and shrubs that are native to warm environmental conditions of Africa and Asia (13). Most species of the genus *Albizia* are used in traditional medicine for the treatment of rheumatism, stomach ache, cough, diarrhoea, wounds, and helminths (14). Also, *Albizia* plants have therapeutic uses in traditional Indian and Chinese medicine for insomnia, irritability, wounds, as antidysentric, antiseptic, and anti-TB agents (14).

*Albizia anthelmintica* has been used by pastoralists throughout East Africa to treat helminth parasitosis (12), as well as controlling helminth parasites in both human and animal medicine in Sudan, Ethiopia and Tanzania (15). The roots and bark are used as a remedy to treat intestinal worms especially in small stock (sheep and goats) (16). In addition, small branches are used as tooth brushes for oral hygiene (16). The root and stem bark of this plant is used to cure symptoms of malaria and herpes (17). Traditional healers in Dar es Salaam, Tanzania, boil the leaves of *A. anthelmintica* in water and administer the drink to epileptic patients (13).

The present study looked at the antibacterial and antioxidant properties as well as investigated the phytochemical composition of leaves, roots and stem bark ethanol extracts of local flora of *A. anthelmintica.* There are no reports in the literature on the antibacterial and phytochemical analysis of the plant species, and no antioxidant studies have been reported.

## 1.2 Statement of the problem

*Albizia anthelmintica* is used traditionally as an antimicrobial agent in both humans and animals, but there is no literature available to support the antimicrobial uses of this plant. Also, there is a need for safer and better antioxidants since current anti-oxidative drugs have safety concerns (18). In addition, oxidative stress is linked to biological damage which could lead to the development of various diseases such as cancer, arthritis, autoimmune disorder, cardiovascular and neurodegenerative diseases (19). There are no results found in the literature for antibacterial studies done on the plant species, nor are there reports found on the study of antioxidant properties. In addition, the literature search for phytochemical studies of the plant yielded minimal results. The total phenolics and flavonoids content present in *A. anthelmintica* is unknown and needs to be determined, as these two chemical compounds are known to possess good antioxidant and antibacterial properties.

## 1.3 Objectives of the study

The objectives of the study were to:

- Determine the antibacterial activity of leaves, roots and stem bark ethanolic extracts of *Albizia anthelmintica* against pathogenic bacteria; *Alcaligenes faecalis* (ATCC 8750), *Shigella sonnei* (ATCC 25931), *Enterococcus faecalis* (ATCC 7080), *Serratia marcescens* (ATCC 8100) and *Staphylococcus aureus* (ATCC 25923).
- Evaluate the antioxidant properties of *Albizia anthelmintica* extracts using three antioxidant assays namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) phosphomolybdenum reduction and ferric reducing antioxidant power (FRAP) assays,
- Determine the total phenolic and flavonoids content of *Albizia anthelmintica* extracts.

- Screen the extracts for the presence of alkaloids, flavonoids, saponins, antraquinones, tannins, terpenoids, glycosides and phenols.

### 1.4 Significance of the study

The study of plants as antibacterial agents is important since most bacteria species have developed resistance against existing agents. Therefore, the present study will contribute to the on-going search for new antibacterial agents (3). In addition, evaluating natural sources for antioxidant activity could lead to the discovery of safer and better antioxidants which could be administered as herbal supplements. The findings of this study will add value to the uses of *Albizia anthelmintica* and investigating this plant for antibacterial and antioxidant properties as well as determining the presence of any major compounds that might be responsible for the biological activities will aid in validating its traditional uses.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. A review of *Albizia* species

*Albizia* species belong to the *Fabaceae* family (16). The genus consists of approximately 150 species of which most are deciduous woody trees and shrubs (20). Different *Albizia* species such as *A. procera*, *A. lebbeck*, *A. julibrissin* and *A. amara* are of great value in ayurvedic medicine (20). In Africa, members of the genus *Albizia* are used in folk medicine in treating rheumatism, coughs, diarrhoea and injuries (21). *Albizia* species are known to be rich in phenolics and terpenes in different plant organs (22) and phytochemical investigation on different *Albizia* species revealed the presence of different classes of secondary metabolites such as saponins, terpenes, alkaloids, flavonoids, triterpenoids, diterpenoids lignans and pyridine glycosides (14). Various *Albizia* species reportedly possess various biological activities, as they have demonstrated antioxidant, antidiabetic, antihelmintic, antibacterial, anti-inflammatory, and hepatoprotective activities (14) and an exploration of the literature revealed that *Albizia* plants have remarkable medicinal value (13).

### 2.1.1 *Albizia procera*

The bark of *Albizia procera*, a tree cultivated in public gardens of Egypt (21) is used in folk medicine to relieve symptoms of stomach and intestinal diseases (22, 23). All plant parts of *A. procera* have reportedly shown anticancerous activity, and a bark decoction is administered to individuals with haemorrhage and rheumatic. The bark of *A. procera* reportedly has antiarthritis and antioxidant activity (23) and five triterpenes have been isolated from the bark of *A. procera* (21). Also, according to a study by Khatoon *et al* (24), bark extracts have reportedly shown anti-

HIV activity whereas bark and leaf extracts have displayed DPPH free radical scavenging activity. In addition, the leaves of *A. procera* are used to treat ulcers (24).

### 2.1.2 *Albizia antunesiana*

*Albizia Antunesiana,* one of the important plants in Zimbabwe's folk medicines has several medicinal uses. The bark extracts of this plant are consumed as a constipation remedy whereas the leaf extract is drunk as a purgative remedy and a remedy prepared from root extract is taken by individuals suffering from diarrhoea and gonorrhoea (11). Also, a root remedy is drunk by infertile women to enhance fertility (25). *Albizia antuneisana* has been explored for antioxidant activity in a study by Chipiti *et al* (26). Antioxidant activity using reducing power ($Fe^{3+,}$ $Fe^{2+),}$ 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and nitric oxide radical scavenging methods has been reported for *A. antunesiana* and several aromatic phenolic compounds, a coumarin and some common tri-terpenoids have also been reported for root and leaf extracts of the plant (26).

### 2.1.3 *Albizia julibrissin*

*Albizia julibrissin* is an umbrella – shaped tree distributed across Africa, China, Mid Asia, East Asia and North America (27, 28). Flowers and bark of *A. julibrissin* are used by the Chinese to cure burns, bruises, abscesses, ulcers, boils, haemorrhoids and fractures (29, 30). In Asia, dried stem barks of *A. julibrissin* are boiled with water and the soup is administered to treat insomnia, diuresis and confusion (31). Studies have revealed the presence of saponins, julibrosides, phenolic compunds and lignans in the stem bark, flowers and leaves of *A. julibrissin* (32).

### 2.1.4 *Albizia lebbeck*

*Albizia lebbeck* is a tree native to tropical Southern Asia (33) and is amongst the most common *Albizia* species world-wide (13). The stem bark of *A. lebbeck* is used in ayurvedic system of

medicine to treat diarrhoea, edema, poisoning, asthma and bronchitis (34). Decoctions prepared from leaves and barks of *A. lebbeck* are reportedly protective against bronchial asthma and allergies. In addition, barks are used to relieve toothache and treat gum diseases (35). Furthermore, the flowers are traditionally used in Chinese medicine for the treatment of anxiety, depression, and insomnia (13). The bark of *A. lebbeck* reportedly contains catechins, kaempferol, quercetin, saponins, triterpenoids and glycosides (36). Studies of *A. lebbeck* leaf extracts revealed the presence of alkaloids, steroids, saponins, terpenoids, glycosides and flavonoids (37).

### 2.1.5 *Albizia amara*

*Albizia amara* is a medicinal plant that occurs throughout southern India(38). All plant parts of *A. amara* are reportedly important in traditional medicine (39) and have several uses in folk medicine for treating diarrhoea, gonorrhoea, skin diseases, leprosy and poisonous bites (40). The leaves and flowers of *A. amara* are applied to swellings, boils and eruptions as an emetic. In addition, leaves and flowers are used as a remedy for malaria, coughs, ulcers and dandruff (41). *Albizia amara* has been evaluated for its phytochemical constituents and studies have revealed the presence of alkaloids, saponins, phenolic compounds, glycosides, tannins and sterols (40). The plant reportedly has anticancer, anti-hyperlipidemic, analgesic, antimicrobial, anti-inflammatory and antioxidant activities (40).

**Table 1: Various compounds isolated from some *Albizia* species**

| Species | Plant parts | Phytocontituents |
|---|---|---|
| ***A. subdimidiata*** | Whole plant<br>Bark | Albiziatrioside A and B<br>Julibroside J29**,** J30 and J31 |
| ***A. julibrissin*** | Flowers | quercitrin and isoquercitrin<br>3, 5, 4'-trihydroxy, 7, 3- dimethoxy-3-*O*-β-D-glucopyranosyl- α-L-xylopyranoside |
| ***A. grandibracteata*** | Leaves | Grandibracteosides A−C |
| ***A. procera*** | Bark | 3-O-[β-Dxylopyranosyl-(1→2)-α-L-arabinopyranosyl- (1→6)-2 acetamido-2- deoxy-β-Dglucopyranosyl] echinocystic acid, 5,2', 4'-trihydroxy-3,7,5'- trimethoxyflavonol-2'-O- β-D-galactopyranosyl- (1→4)-O-β D-glucopyranoside |
| ***A. chinensis*** | Bark<br>Leaves | Albizosides A-C<br>Kaempferol-3-O-α-L-rhamnopyranoside, Quercetin-3-O-α-L-rhamnopyranoside, Luteolin, Kaempferol, Quercetin |
| ***A. gummifera*** | Bark | Vitalboside-A, vitalboside-A, 2'- methylglucuronate 3-O-{β-D-glucopyranosyl(142)-[α-L-arabinopyranosyl(146)]-β-D-glucopyranosyl}-oleanolic acid |
| ***A. lebbeck*** | Seeds | Budmunchiamines L1-L3. |

| | | |
|---|---|---|
| | Leaves<br>Bark | Quercetin, kaempferol, 3-*O*-α-rhamnopyranosyl (1→6)-β-glucopyranosyl(1→6)-β-galactopyranosides.<br>Albiziasaponins A, B and C |
| ***A. myriophylla*** | Bark<br>Stem | Albizzine A<br>Albiziasaponins A-E |
| ***A. inopinata*** | Leaves | Felipealbizine A, felipealbizine B |
| ***A. versicolor*** | Whole plant | Lupeol**,** acacic acid, lactone |
| ***A. mollis*** | Bark | Molliside A-B, Concinnoside A, Albiziasaponin A |
| ***A. odoratissima*** | Root bark | 7,8-Dimethoxy-39,49 methylenedioxyflavone, 7,29,49-Trimethoxyflavone |
| ***A. falcataria*** | Bark | Syringaresinol |

Source: Indravathi *et al*, (40)

**2.2 Description and geographical locations of *Albizia anthelmintica***

*Albizia anthelmintica*, which is the plant under study, is commonly known as "worm-cure" *Albizia* in English, *oumaboom* in Afrikaans, and *omupopo* in Oshindonga (16). *Albizia anthelmintica* is widely distributed in tropical Africa, Namibia and Swaziland. In Namibia, the plant is widely distributed except in the extreme South and Namib dessert. *Albizia anthelmintica* is a small multi-stemmed tree. Its leaves are almost circular and have 2-4 pairs of pinnae. The plant has creamy-white flowers that are contained in fluffy, semi-spherical heads and flattened pale brown pods (16).

**Figure 1**: *Albizia anthelmintica* (local flora). Picture was taken during sampling.

### 2.3 Traditional uses and studies done on *Albizia anthelmintica*

*Albizia anthelmintica* is commonly used in Namibia by traditional healers in Rundu, and Kavango East region (42). The bark is crushed, mixed with water and the infusion is administered orally to malaria patients (42). Not only is the plant used to cure malaria in Namibia, but similar uses of the plant have been reported in Ghana and Zambia (43). Traditional healers in northern Botswana use the roots of *A. anthelmintica* to manage HIV/AIDS related opportunistic infections which includes symptoms of persistent coughs, diarrhoea, skin rashes, tuberculosis, frequent fevers, sores, thrash and womb problems (44). The poor small stock farmers and pastoralists in East Africa use *A. anthelmintica* to treat their livestock against internal parasites (45). *Albizia anthelmintica* has been investigated for anti helminth properties

against naturally occurring infections of mixed gastrointestinal parasites in Ugandan sheep, whereby the plant was found to be active against gastrointestinal nematodes (12). Grade *et al* (12) reported the presence of triterpenoid saponins, histamine, tannins, and other phenolic compounds in the bark of *Albizia anthelmintica*. Amongst the compounds reportedly present in the bark of *A. anthelmintica*, tannins, alkaloids and phenolic compounds have been reported for antihelminth activity (8).

### 2.4 Natural phytochemicals as a source of medicine

Medicinal plants can be defined as plants that have at least one of their parts utilized for therapeutic purposes (46). Plant extracts, either in the form of pure compounds or standardized extracts, have an unmatched phyto- component diversity (30). Plants with medicinal uses possess bioactive compounds, which can be defined as secondary metabolites naturally present in different plant parts such as leaves, flowers, vegetables, and roots. Each bioactive compound has specific functions but the major function is defence and protection of plants against various diseases (47, 48). Medicinal plants produce a variety of phytochemicals such as flavonoids, alkaloids, tannins, and glycosides with a wide range of applications including medicinal uses (49). Due to the phytochemical contents of medicinal plants, numerous herbal plant species reportedly show an important role in the development of new medicine (50). This has led to a tremendous increase in research on natural phytochemicals as a source of medicine (49).

### 2.5 Natural Products as antioxidants

Antioxidants are any substances that significantly delay or inhibit oxidation of a substrate (lipids and other biomolecules), by preventing initiation of oxidizing chain reactions by radicals or through quenching the propagation of those chain reactions (51, 52). Antioxidants are divided into two categories, on the basis of their reaction mechanisms, and these are hydrogen atom

transfer (HAT) and electron transfer (ET) (53). HAT applies to antioxidants with an ability to quench free radicals by hydrogen donation whereas ET applies to antioxidants that have an ability to transfer one electron to reduce any compound including radicals, metals and carbonyls (53). Free radicals are constantly generated in all living cells as part of normal cellular functions (54). However, An excess of reactive/oxidizable species can result in damages of cellular lipids, deoxyribo nucleic acids (DNA) and proteins by means of oxidative actions, and this might result in loss of cellular function or even cellular death (55).

Free radicals are produced either as end products of an individual's metabolic processes, or directly by xenobiotics (56). However, the oxidative stress that result from excess/accumulation of free radicals in cells can be neutralized or scavenged by endogenous or exogenous antioxidants (57). Endogenous antioxidants include enzymes (e.g superoxide dismutase, glutathione peroxidases, and catalases), extracellular proteins that bind iron and copper (e.g albumin, ceruloplasmin, and haptoglobin), antioxidant vitamins (e.g beta carotene, vitamin C) and other cellular compounds (e.g quinones and bilirubin) (58).

Synthetic antioxidants (which form part of exogenous antioxidants), such as synthetic phenolic antioxidants (SPAs) are the most frequently used in a wide range of products that are used on a daily basis (59). Food products such as butter, cooking oil, margarine and cheese are amongst the those that contain synthetic antioxidants (60). In addition, many synthetic phenolic antioxidants such as t-butyl-4-hydroxyanisole (BHA) and t-butylhydroquinone are used as additives in cosmetics (e.g body creams and lotions, lipstick) (61, 62). Synthetic antioxidants are considered to be safe for human health, with exposure to a certain level (59). However, there are controversial toxicological data on synthetic antioxidants that have drawn attention for a search

of alternative sources of antioxidants (59) and currently there is an emphasis on replacing the use of synthetic antioxidants with those derived from natural sources (63).

Natural antioxidants, especially phenolics and flavonoids are considered to be safe, and this has led to a great interest in finding new antioxidants from natural sources (22). In addition, there is a lack of documented evidence indicating adverse effects of natural antioxidants (58) and they are also reported to be very beneficial to health, as they protect against coronary heart diseases, cancer, and hypertension (64).

### 2.6 Natural Products as antibacterial agents

Antibacterial agents are substances that act against bacterial infections by killing the bacteria or inhibiting their growth (65). Antibacterial agents target bacterial DNA, interfere with bacterial cell wall synthesis and function or attack bacterial metabolic processes such as protein synthesis (65). Emergence and re-emergence of lethal infectious diseases such as influenza viruses, cholera, and hepatitis B caused by pathogenic microorganisms is considered to be a world-wide crisis (66, 67). Over the years, antimicrobial resistance of bacteria has been on the rise, which has resulted in the treatment of bacterial infections being difficult and complicated by antibiotic resistance (68). Also, there has been many health problems associated with currently available synthetic antimicrobial agents, hence a need of discovering alternative new, broad spectrum , more active and safer antimicrobial agents (67).

The ability of bacteria to develop resistance against antimicrobial agents may be acquired by de novo mutation or via an acquisition of resistant genes from other organism (68). Bacteria resistance could be through producing enzymes that destroy the antibacterial drug, developing efflux systems that prevent the drug from reaching its intracellular target, modification of the

drug's target site, and producing an alternative pathway that bypasses the action of the drugs (68).

Most infectious diseases caused by pathogenic fungi, viruses and bacteria have been treated with plant extracts (69), and plant materials remain an important natural source in combating various diseases around the globe (67).The ability of plants to synthesize de novo antimicrobial agents in response to microbial attack has led to the exploration of medicinal plants in hope for the development of new antibacterial drugs (67). Natural drugs could be original natural products, synthetic products based on natural plant structures or products derived or chemically synthesized from natural sources (70). Pefloxacin, norfloxacin, ciprofloxacin and levofloxacin are examples of antibiotics of natural origin which were derived from an alkaloid quinine, an active compound of the plant *Cinchona succirubra* (70).

### 2.7 Test bacteria

In this study, antibacterial activity of leaf, root and bark extracts of *Albizia anthelmintica* was evaluated against pathogenic strains of *Staphylococcus aureus* (ATCC 25923), *Serratia marcescens* ( ATCC 8100), *Shigella sonnei* (ATCC 25931), *Alcaligenes faecalis* (ATCC 8750) and *Enterococcus faecalis* (ATCC 7080).

#### 2.7.1 *Staphylococcus aureus*

*Staphylococcus aureus* (strain ATCC 25923) is a gram positive bacteria well known as methicillin resistant *Staphylococcus aureus* (MRSA), due to its resistance to semi synthetic penicillin (a beta-lactam antibiotic) and many other antimicrobial agents (71). *Staphylococcus aureus* resistance to methicillin is due to an additional penicillin-binding protein( PBP2), which has a very low affinity for beta – lactam agents (71). According to Nobandegani *et al* (72),

antimicrobial resistance of MRSA strain has raised concerns due to reports on the bacterium being associated with worse clinical outcomes, such as respiratory infections of cystic fibrosis patients and the strain has been identified to be among the causative agents of cystic fibrosis, especially in small children. In addition, methicillin resistant *Staphylococcus aureus* is associated with infections of the central nervous system, skin, bones and joint infections (73).

### 2.7.2 *Serratia marcescens*

*Serratia marcescens* is a gram negative bacterium that frequently exhibit resistance to a broad-spectrum of beta lactams (74). Beta lactams are antibiotics such as penicillin that contain a beta lactam ring in their structure (75). Resistance of *Serratia marcescens* to beta lactams is due to its ability to produce an excess of the chromosomal AmpC enzyme (an enzyme that mediates antibiotic resistance) and acquiring plasmid borne extended-spectrum beta – lactamases (74, 76). Most isolates of *S. marcescens* are considered a threat to human health, as they cause illnesses that are challenging to treat due to multidrug resistance of the bacteria (77). *Serratia marcescens* reportedly causes respiratory tract, urinary tract and surgical wound infections and is a causative agent of meningitis in paediatricians (78).

### 2.7.3 *Shigella sonnei*

*Shigella sonnei* is a gram negative, rod shaped and non-spore forming bacterium; ranked third in the United States amongst bacterial food borne pathogens according to the Centres for Disease Control and Prevention (79, 80). *Shigella* species are the causative agents of shigellosis, an invasive infection of the human colon characterized by fever, vomiting, bloody diarrhoea and intestinal cramps (81). Shigellosis outbreaks may result from contaminated raw foods or inappropriately prepared processed food and disease spreads rapidly due to its ease of transmission from person to person, via the faecal-oral route (79).

### 2.7.4 *Alcaligenes faecalis*

*Alcaligenes faecalis* is an anaerobic gram negative bacterium. Most strains of this bacteria display multi-drug resistance to numerous antibiotics, especially beta lactams, quinolones and aminoglycosides (82). *Alcaligenes faecalis* occur in the alimentary tract as a harmless saprophyte and systematic infections by these bacteria are very uncommon (83). However, *A. faecalis* has been associated with causes of meningitis, enteric fever, sepsis, and pneumonitis and infections by *A. faecalis* may be fatal due to resistance of the bacteria to most antibiotics (83).

### 2.7.5 *Enterococcus faecalis*

*Enterococci* are ubiquitous bacteria that are capable of surviving extremes of temperature, pH and high salt concentrations (84). *Enterococci* species are the most thermo-tolerant non-sprouting bacteria and can survive harsh conditions during food preparation (cooking and processing), specifically in meat (84). *Enterococci* bacteria occur naturally in the gastrointestinal tract of humans and other animals, but can cause systematic opportunistic infections (85), as well as oral infections that are difficult to treat, due to multidrug resistance (86). In addition, recent studies have found *E. faecalis* to be one of the main cause of human urinary tract infections (UTI) (87).

## 2.8 A review of the methods employed

Evaluating local ethno medicinal flora for various biological activities is a driving force that leads to the isolation and characterization of the active compounds contributing to drug discovery (7). In the present study, antioxidant activity was determined by means of DPPH, FRAP and phosphomolybdenum methods, whereas antibacterial activity was evaluated using the disc agar diffusion method and agar dilution method was used to determine minimum inhibitory concentration (MIC) values.

### 2.8.1 Antioxidant assays

#### (a) 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH is a purple coloured stable free radical that has an unpaired valence electron at one atom of nitrogen bridge (88). DPPH dissolves best in ethanol or methanol, and its absorbance is usually measured at a wavelength of about 515 – 520 nm (52, 88, 89). The DPPH method is usually chosen for antioxidant analysis because it is an easy and fast method (52). In the presence of an antioxidant, the free electron is paired and the colour of DPPH changes to yellow and the colour change can be used spectrophotometrically to determine the antioxidant activity of compounds (90).

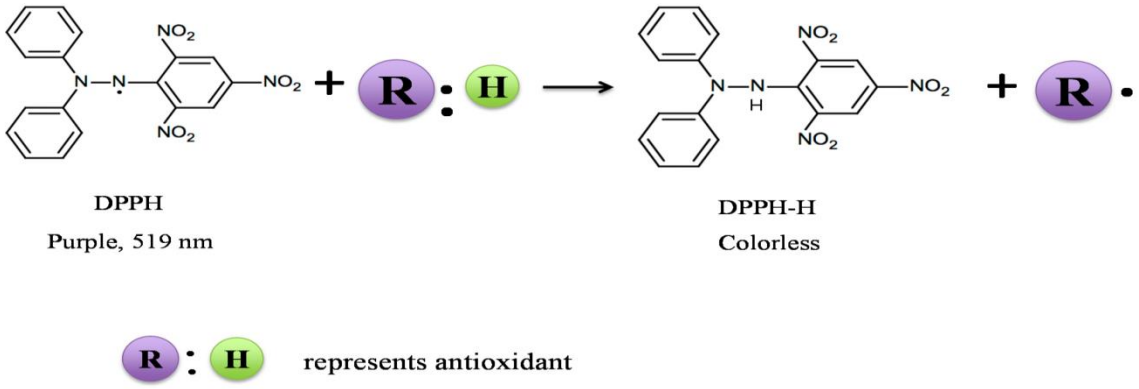


**Figure 2**: A general reaction mechanism of DPPH with an antioxidant.

Source: Liang & Kitts, (91)

**(b) Ferric reducing antioxidant power (FRAP) assay**

FRAP is a simple, rapid, inexpensive and robust assay that does not require specialized equipment (92). The assay is based on the ability of compounds to reduce yellow ferric tripridyl triazine complex (Fe $^{3+}$-TPTZ) to a blue ferrous tripridyl triazine complex (Fe $^{2+}$-TPTZ) by the action of electron-donating antioxidants (92). The absorbance of the resulting blue colour of the ferrous complex is measured spectrophotometrically, usually at a wavelength in the range of 593-700 nm (93-95). The absorbance is taken as directly related to the total reducing capacity of electron-donating antioxidants (96).

**(c) Phosphomolybdenum assay**

Determination of total antioxidant capacity is a spectroscopic based procedure for the quantitative analysis of antioxidant capacity of a sample analyte (97).The phosphomolybdenum method is based on the reduction of Mo(VI) to a green phosphate Mo(V) complex by the sample under investigation at an acidic pH (98, 99). The absorbance is measured at a wavelength of about 695-700 nm (18, 100). The reduction of Mo(VI) to Mo(V) usually occurs in the presence of antioxidants such as ascorbic acid, some phenolic compounds, carotenoids and alpha tocopherol (101).

**2.8.2 Antibacterial activity screening assays**

**(a) Disc agar diffusion method**

The disc agar diffusion susceptibility method is one of the various qualitative methods used for antibacterial activity screening. The method is simple and practical and has been well standardized (102). The test is done by inoculating a bacterium sample onto the surface of an agar plate and paper discs impregnated with specific volumes and concentrations of antibiotics or

extract samples are placed on the inoculated agar, and the clear zones around the discs are measured to the nearest milimeter (mm) (102).

**(b) Minimum inhibitory concentration**

There is a lack of quantitative standard methods of antimicrobial screening. However, there are a few semi-quantitative procedures in place, and minimum inhibitory concentration (MIC) is one of them. MIC is defined as the lowest concentration of an antimicrobial agent which inhibits visible microbial growth after 24 hours of incubation (103). The test is done by placing a test level of an inoculum and the extract sample into tubes containing culture broth. The end result is determined by observing the tubes with no growth by means of measuring turbidity, and the least concentration where no visible growth was observed is interpreted as the MIC (104).

## CHAPTER 3

## METHODS

### 3.1 Research ethics

A research/plant collection permit was obtained from the Ministry of Environment & Tourism (permit number: 1992/2014); and an ethical clearance certificate was issued by the University of Namibia (UNAM) research and publications office (ethical clearance reference number: FOS/97/2016). The plant from which the samples were obtained was not uprooted, and small quantities of root samples were collected in order to prevent the plant from dying. In addition, the plant under study is not amongst the endangered plant species. A sample of *Albizia anthelmintica* with a specimen number TN01/02 was press-dried following procedures provided by the National Herbarium of Namibia (WIND). Identification of the specimen was undertaken by Mr Levi Nanyeni at WIND.

### 3.2 Research design

The study was conducted as summarised in the diagram below

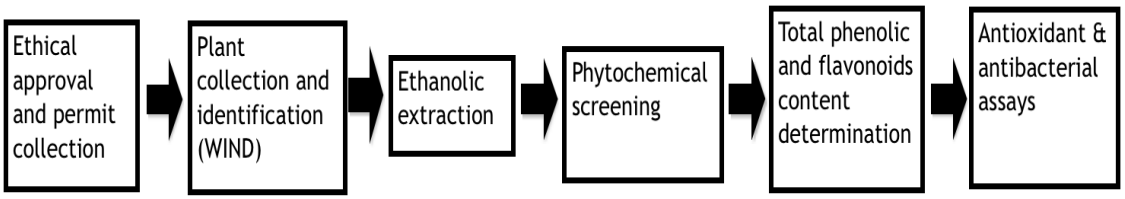


**Figure 3:** A summary of the study methods.

### 3.3 Plant material

Samples of fresh leaves, roots and stem bark plant samples of *Albizia anthelmintica* were manually collected in Ovamboland (Oshana) region; Namibia, because they are easily accessible in this region. The samples were air dried for three weeks at room temperature, away from direct sunlight.

### 3.4 Preparation of crude extracts

Plant parts were powdered by means of a blender and steeped in ethanol (20 g of sample per 200 mL ethanol) and the flasks were left on an orbital shaker set at 100 rpm for 48 h to allow for efficient extraction. After extraction, the macerates of each sample were filtered into separate flasks by means of Büchner filtration and using Whatman no 4 filter paper. The residues after the filtration process were discarded and the filtrate was concentrated using a rotary evaporator set at 40ºC and transferred to pre-weighed vials to dry at room temperature. After drying, the masses of the dry extracts were determined and the extracts kept safe at room temperature until usage.

### 3.5 Phytochemical screening

#### 3.5.1 Qualitative Phytochemical screening

Preliminary phytochemical screening was carried out following standard procedures previously described by Tiwari *et al* (8) for alkaloids, saponins and terpenes whereas screening for the presence of flavonoids, phenols, tannins, glycosides and anthraquinones was done using methods by Geetha *et al* (105).

**(a) Alkaloids**

**Mayer's Test:** Ten mg of extract was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow precipitate indicated the presence of alkaloids.

**Dragendroff's Test:** Ten mg of extract was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of a red precipitate indicated the presence of alkaloids.

(**b) Flavonoids**

**Shinoda Test**: Ten mg of extract were added to a pinch of magnesium turnings followed by 2 drops of concentrated hydrochloric acid. Formation of a pink colour indicated the presence of Flavonoids.

**(c) Tannins**

**Ferric chloride test**: Five percent of ferric chloride (0.5 mL) was added to 5 mg of extract. The development of a dark bluish black colour indicated the presence of tannins.

**(d) Phenols**

**Sodium hydroxide test**: Five mg of extract were dissolved in 0.5 mL of 20% sulphuric acid solution, Followed by addition of few drops of aqueous sodium hydroxide solution. If the mixture turned blue it indicated the presence of phenols.

**(e) Glycosides**

The extract (0.5 mg) was dissolved in 1 mL of water and then 1 M aqueous sodium hydroxide solution was added until formation of yellow colour which indicated the presence of glycosides.

**(f) Saponins**

**Foam Test:** Extract (0.5 mg) was shaken with 2 mL of distilled water. If the foam persisted for ten minutes, the test is positive.

**(g) Diterpenes**

**Copper acetate Test:** Extracts (0.5 mg) were dissolved in water and treated with 3-4 drops of 5% copper acetate solution. Formation of emerald green colour indicated the presence of diterpenes.

**(h) Anthraquinones**

**Borntragers test:** The extract (0.5 mg) was placed into a dry test tube followed by addition of 5 mL of chloroform and shaken for 5 min. The extract was then filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicated the presence of anthraquinones.

**3.5.2 Quantitative phytochemical screening**

Total phenolic and total flavonoid contents were determined using previously described procedures by Marinova *et al* (106) with minor modifications.

**(a) Analysis of total phenolic content**

Total phenolic content of the different extracts was determined using the Folin- Ciocalteu method. Extracts were tested at a concentration of 0.40 mg/mL. One mL of extracts dissolved in 70% ethanol was added to a 25 mL volumetric flask containing 9 mL of distilled water. Folin Ciocalteu phenol reagent (1 mL) was added to the mixture and shaken. The mixture was allowed to stand for 5 min where after 10 mL of 7% sodium carbonate solution was added and the solution was diluted to volume (25 mL) with distilled water. The mixture was incubated for 90 min at room temperature and the absorbance against prepared reagent blank was determined at 750 nm using a spectra max M2 spectrophotometer (molecular devices, USA). A reagent blank

was prepared using distilled water. Gallic acid served as the positive control at concentrations of 0.63, 1.25, 2.50, 5.00, 10.00 mg/mL. Gallic acid standard curve of absorbance vs concentration was constructed using Microsoft excel.

**(b) Analysis of total flavonoids content**

The total flavonoids content (TFC) was determined by means of aluminium chloride colorimetric assay. Extracts were tested at a concentration of 0.40 mg/mL of 70% ethanol. One mL of the extract was added to a 10 mL volumetric flask containing 4 mL of distilled water. To the flask, 0.3 mL of 5% sodium nitrate solution was added and the mixture was incubated for 5 min at room temperature. After incubation, 0.3 mL of 10% aluminium chloride was added and the mixture incubated at room temperature for 6 min. Two mL of 1 M sodium hydroxide was added and the total volume was made up to 10 mL, using distilled water. The solution was mixed well and the absorbance was measured against a prepared blank reagent at 510 nm using a spectra max M2 spectrophotometer (molecular devices, USA). Quercetin was used as a standard control at concentrations of 0.63, 1.25, 2.50, 5.00, 10.00 mg/mL. Quercetin standard curve of absorbance vs concentration was constructed using Microsoft excel.

### 3.6 Determination of antioxidant activity

#### 3.6.1 DPPH free radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was measured according to a modified protocol previously described by Kapewangolo *et al* (107). The extracts dissolved in ethanol were mixed with an ethanol solution of DPPH (90 µM), then incubation of extract with DPPH in the dark at room temperature was carried out for 30 min. The absorbance values were measured at 520 nm using a spectra max M2 spectrophotometer (molecular devices, USA), and

converted into percentage inhibition antioxidant activity. A known antioxidant, ascorbic acid, was used as a standard control.

### 3.6.2 Ferric reducing antioxidant power assay

The reducing power of the extracts was evaluated according to a protocol previously described by Ferreira *et al* (18). Extracts were tested at varying concentrations of 0.16, 0.31, 0.63, 1.25 and 2.50 mg/mL of 70% ethanol. Extracts (2.5 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, followed by an addition of 2.5 mL of 10% trichloroacetic acid (w/v), and subsequently centrifuged at 650 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured at 700 nm using a spectra max M2 spectrophotometer (molecular devices, USA). Gallic acid was used as a standard control.

### 3.6.3 Determination of total antioxidant capacity

The total antioxidant capacity (TAC) of the extract was evaluated using the phosphomolybdenum method by Rhumzum *et al* (7). Varying concentrations of extracts (0.06, 0.13, 0.25, 0.50, and 1.00 mg/ mL of 70% ethanol) were tested. Extracts (0.3 mL) were combined with 3mL of phosphomolybdenum reagent solution. The tubes containing the reaction solution were incubated at 95°C for 90 min and cooled to room temperature, then the absorbance of the solution was measured at 695nm using a spectra max M2 spectrophotometer (molecular devices, USA) against a blank. Methanol (0.3mL) was used as the experimental blank whereas ascorbic acid was used as a standard control.

### 3.7 Antibacterial activity assay

The determination of antibacterial activity was conducted by employing modified methods previously described by Seyydnejad *et al* (108) and Obiajunwa *et al* (3). All apparatus used for antibacterial activity assays were sterilized in an autoclave machine at 121ºC for 30 min, to avoid any form of contamination. In addition, all assays were conducted using aseptic techniques and all apparatus were sterilized after use. Bacteria samples were disposed according to the regulations of the University of Namibia.

#### 3.7.1 Antibacterial activity screen test

An antibacterial activity screen test was conducted to determine whether the extracts were active against the test bacteria at a given extract concentration prior to testing activity at lower extract concentrations. Extracts dissolved in sterile distilled water were screened at one concentration of 10 mg/mL. Each bacterium sample (400 µL) was inoculated onto the surface of nutrient agar. Sterile paper discs were placed onto the inoculated agar using flame sterilized biceps and paper discs were impregnated with 10 µL of the extract. Ampicillin (10 µg/µL) served as the positive control and sterile distilled water as a negative control. The plates were inverted and allowed to dry for an hour in a fridge then incubated for 24 h at 37ºC. The clear zones around the discs were an indication of antibacterial activity and microorganisms that were inhibited by the extracts were used for the agar disc diffusion antibacterial test.

#### 3.7.2 Test microorganisms

A total of five bacterial species were tested, and these were: *Staphylococcus aureus* (ATCC 25923), *Serratia marcescens* (ATCC 8100), *Enterococcus faecalis* (ATCC 7080), *Shigella sonnei* (ATCC 25931), and *Alcaligenes faecalis* (ATCC 8750). Bacterial cultures were prepared

by dissolving a few crystals in sterile nutrient broth. The culture was incubated for 36 h at 37ºC to allow for bacterial growth. The grown culture was kept at 5ºC until further usage.

### 3.7.3 Disc agar diffusion method

Dry extracts were dissolved in sterile distilled water, to prepare solutions of 0.63, 1.25, 2.50, 5.00, and 10.00 mg/mL. A 24 h bacterial suspension (400 µL) was aseptically inoculated on nutrient agar plates. Paper discs (6.00 mm) were placed onto the inoculated plates, and impregnated with 10 µL of prepared extracts of varying concentrations. Sterile distilled water was used as a negative control and the antibiotic Ampicillin (10 µg/µL) was used as the positive control. The plates were inverted and the discs allowed to dry for an hour in a fridge to allow for pre-diffusion of the samples then incubated for 24 h at 37°C. Positive results were established by the presence of clear zones of inhibition around extracts inhibiting bacterial growth. This was measured with a meter ruler and diameters were recorded based on mm.

### 3.7.4 Minimum inhibitory concentration determination

The least extract concentration that inhibited visible growth was determined using the bacteria species whose growth was inhibited in the disc agar diffusion assay. A 16 h culture was serially diluted with sterile nutrient broth to achieve a bacterium sample with a dilution factor of $10^{-5}$. To each sterile tube, 100 µL extract (0.63, 1.25, 2.50, 5.00, and 10.00 mg/mL), 200 µL broth and 20 µL of a bacterium sample were aseptically added. The tubes were incubated for 16 h at 37ºC, and a loop full using an inoculating loop was obtained from each tube and aseptically streaked onto nutrient agar plates. The plates were incubated for 16 h at 37ºC. The MIC value was determined as the lowest concentration of the crude extract in broth medium that inhibited the visible growth of the test microorganism(s).

### 3.7.5 Statistical analysis

All experimental analyses were performed in triplicates and three trials were conducted. Antibacterial and antioxidant experimental results are expressed as mean ± standard deviation (SD).Total Flavonoid and phenolic contents were calculated using standard calibration curves obtained from various diluted concentrations of quercetin and gallic acid curves; respectively. The 50% inhibitory concentrations ($IC_{50}$ values) from the antioxidant assays were computed using Graph Pad prism 6 software.

Moreover, a statistical t-test was run on antibacterial and antioxidant data to determine the significance of the biological activity results, at 95% level of confidence. The results are considered to be significant at $p<0.05$.

# CHAPTER 4

# RESULTS

## 4.2 Plant extraction yield

The extract yield obtained for each sample is given below (table 1)

**Table 2**: Crude extracts yielded

| Sample | Mass used for extraction (g) | Mass of dry extract (g) |
|---|---|---|
| **Leaves** | 93.45 | 5.66 |
| **Roots** | 60.25 | 4.68 |
| **Stem bark** | 61.56 | 6.84 |

## 4.3 Qualitative phytochemical data

The preliminary phytochemical screening of the leaves, roots and stem bark was done to appraise the presence of bioactive components. The presence of alkaloids, flavonoids, tannins, phenolics, saponins, and diterpenes was determined while anthraquinones and glycosides were not detected. The results are summarized in table 2.

**Table 3**: Phytochemical constituents of *A. anthelmintica* roots; stem bark and leaves ethanolic extracts.

| Test | Extract | | |
|---|---|---|---|
| | **Roots** | **Bark** | **Leaves** |
| **Alkaloids** | +++ | ++ | - |
| **Flavonoids** | + | + | +++ |
| **Tannins** | + | - | - |
| **Phenolics** | +++ | ++ | + |
| **Anthraquinone** | - | - | - |
| **Saponins** | ++ | +++ | - |
| **Diterpenes** | +++ | ++ | + |
| **Glycosides** | - | - | - |

- Absence; + Slight presence; ++ Medium presence; +++ High presence

### 4.3 Quantitative phytochemical data

#### 4.3.1 Total Phenolic Content

The total phenolic content (TPC) was expressed as gallic acid equivalents (table 3). Significant ($p<0.05$) differences were observed for TPC among the three different extracts, with the highest TPC of 1 741.08 ± 0.05 mg GAE/100 g fresh mass observed for the root extract, and the lowest TPC of 308.64 ± 0.01 for the bark extract.

### 4.3.2 Total Flavonoid Content

The total flavonoid content (TFC) was significantly ($p<0.05$) higher in the root extract compared to the bark and leaf extracts as recorded in quercetin equivalents (table 3). TFC of root, leaf and bark were 366.80, 114.49, and 9.75 mg GAE/100 g fresh sample, respectively.

**Table 4**: Total phenolic and flavonoid contents in leaves, roots and stem bark ethanol extracts of *A. anthelmintica.*

| Extract | TPC (mg GAE/100 g fresh mass) | TFC (mg GAE/100 g fresh mass) |
|---|---|---|
| **Leaves** | 535.05 ± 0.01 | 114.49 ± 0.01 |
| **Roots** | 1 741.08 ± 0.05 | 366.80 ± 0.02 |
| **Stem bark** | 308.64 ± 0.01 | 9.75 ± 0.01 |

## 4.4 Antioxidant activity

### 4.4.1 DPPH radical scavenging activity

The DPPH free radical scavenging activity of leaf, root and bark ethanolic extracts of *Albizia anthelmintica* summarized in figure 3 shows that all three extracts significantly ($p<0.05$) scavenged DPPH free radicals. The $IC_{50}$ values for the leaf, root and bark extracts (table 4) were 0.019 ± 0.01, 0.018 ± 0.01 and 0.024 ± 0.01 mg/mL, respectively. However, the $IC_{50}$ value for the standard ascorbic acid was lower than the lowest concentration (0.020 mg/mL) of ascorbic acid tested.

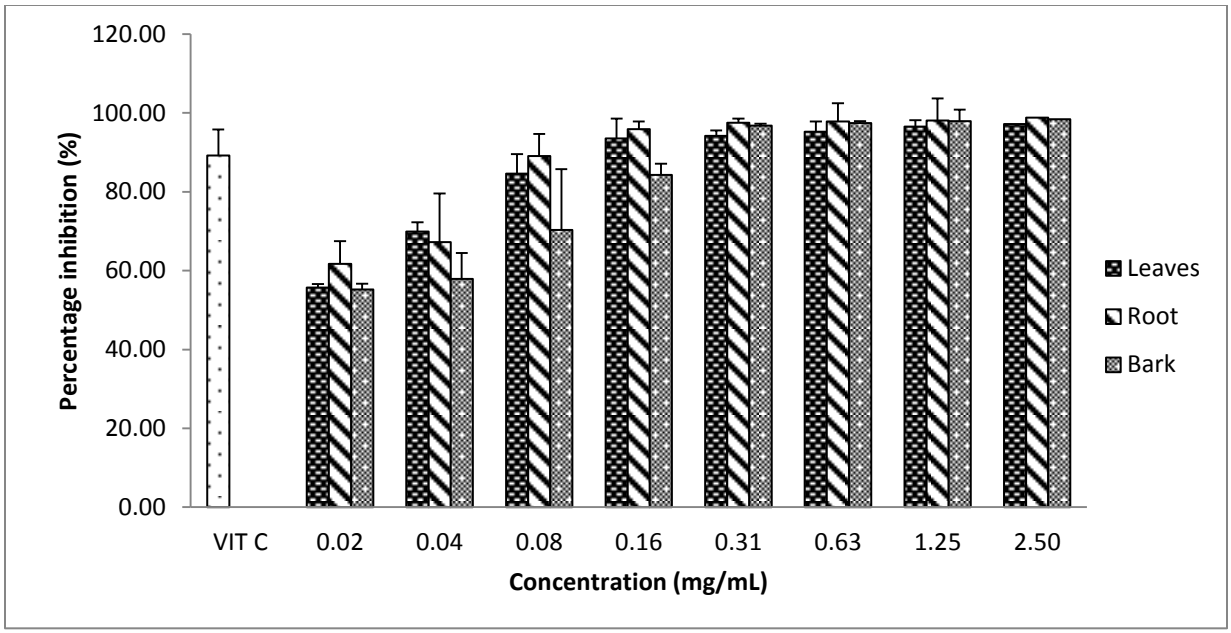


**Figure 4**: DPPH free radical scavenging activity of leaves, roots and stem bark ethanolic extracts of *A. anthelmintica*.

**Table 5**: $IC_{50}$ values of leaves, roots and stem bark ethanolic extract of *A. anthelmintica*

| **Extract** | **$IC_{50}$ value (mg/mL)** |
|---|---|
| **Leaf** | 0.019 ± 0.01 |
| **Root** | 0.018 ± 0.01 |
| **Bark** | 0.024 ± 0.01 |

### 4.4.2 Ferric reducing antioxidant power

All extracts showed an ability to donate electrons to convert $Fe^{3+}$ into $Fe^{2+}$ which is indicated by the concentration dependent increase in the absorbance (figure 3). The yellow colour of the test solutions changed to various shades of green and blue, depending on the reducing power of each extract. A significant ($p<0.05$) reducing power was observed at extract concentration between 1.25 and 2.50 mg/mL for leaves and roots, with absorbance of 0.52 and 0.49, respectively. Bark extract exhibited the least reducing power with an absorbance of 0.06 at a concentration of 2.50 mg/mL. However, the standard gallic acid exhibited greater absorbance values compared to all the extracts, with an absorbance of 0.27 at the lowest concentration (0.16 mg/mL).

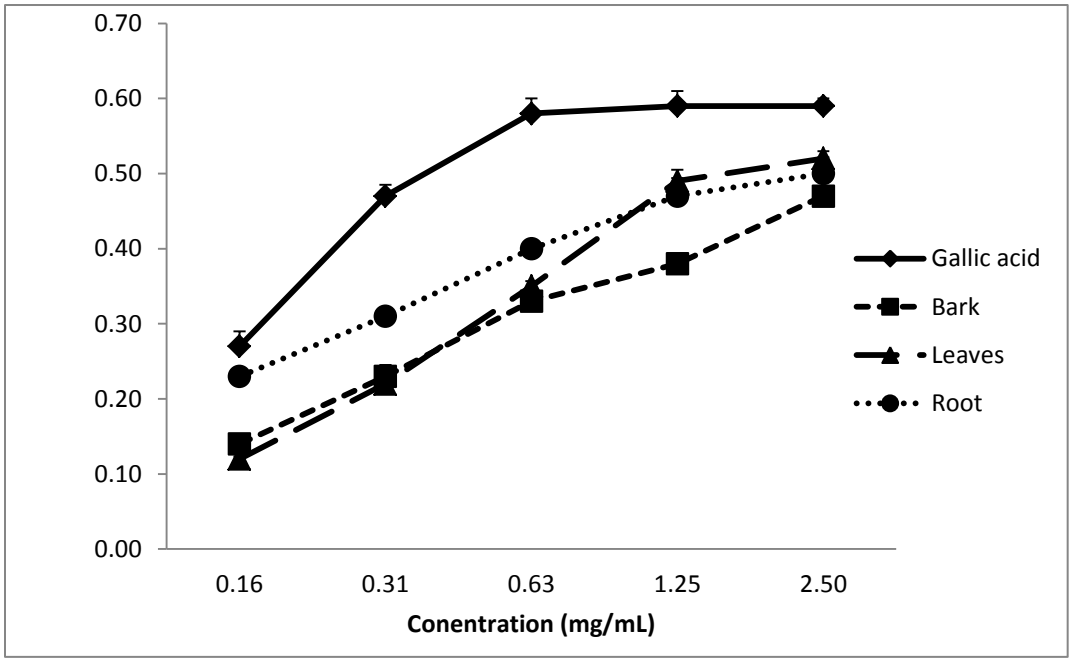


**Figure 5**: Reducing power of ethanolic extracts of *A. anthelmintica* leaves, roots and stem bark.

### 4.4.3 Total Antioxidant Capacity

Total antioxidant capacity (TAC) exerted by the extract is concentration dependent. It was observed that all three extract were likely to have the capacity of reducing Mo (VI) to Mo (V) which was indicated by the formation of a bluish green phosphate/Mo (V) Complex. All extracts exhibited significant reduction ability with the greatest reduction by the roots extract (Abs 0.218 at 1.00 mg/mL). Bark and leaf extracts have an absorbance of 0.171 and 0.127 at 1.00 mg/mL, respectively. The extract absorbance at the highest concentration was greater than those of the ascorbic acid standard (0.117). Table 5 summarizes the TAC of the extracts at various concentrations, in comparison to the standard ascorbic acid.

**Table 6**: Total antioxidant capacity of leaves, roots and stem bark ethanolic extracts of *A. anthelmintica*.

| **Concentration** | **Absorbance at 695 nm** | | | |
|---|---|---|---|---|
| **(mg/mL)** | **Leaves** | **Roots** | **Bark** | **Ascorbic acid** |
| 0.06 | 0.007 ± 0.01 | 0.002 ± 0.01 | 0.009 ±0.00 | 0.069 ±0.01 |
| 0.13 | 0.012 ± 0.01 | 0.018 ± 0.01 | 0.020 ± 0.01 | 0.088 ± 0.01 |
| 0.25 | 0.024 ± 0.01 | 0.039 ± 0.01 | 0.047 ± 0.01 | 0.094 ± 0.01 |
| 0.50 | 0.051 ± 0.01 | 0.112 ± 0.04 | 0.108 ± 0.02 | 0.106 ± 0.01 |
| 1.00 | 0.127 ± 0.09 | 0.218 ± 0.09 | 0.171 ± 0.02 | 0.117 ± 0.01 |

## 4.5 ANTIBACTERIAL ACTIVITY

### 4.5.1 Antibacterial preliminary screening

The test bacteria where subjected to a screen test, and only microorganisms whose growth was inhibited by the extract in the antibacterial screen test were used in the disc agar diffusion method. The growth of all *Alcaligenes faecalis* and *Enterococcus faecalis* bacterial species was not inhibited by any of the extract, and the screen test results are summarized in table 7.

**Table 7**: Antibacterial activity screen test results of leaves, roots and stem bark ethanolic extracts of *A. anthelmintica*

| **Bacteria** | **Extract (10.00 mg/mL) activity** | | |
|---|---|---|---|
| | **Bark** | **Leaf** | **Root** |
| *Staphylococcus aureus* | √ | √ | √ |
| *Serratia marcescens* | √ | √ | √ |
| *Enterococcus faecalis* | × | × | × |
| *Shigella sonnei* | √ | √ | √ |
| *Alcaligenes faecalis* | × | × | × |

× No bacterial growth inhibition √ Bacterial growth inhibited

### 4.5.2 Antibacterial susceptibility data

The antibacterial activity of leaf, root and bark ethanolic extracts of *Albizia anthelmintica* against the five bacterial strains examined were assessed by the presence or absence of zones of

inhibition. The antibacterial activity varied amongst the different extracts, the leaf extract mostly inhibited the growth of *Staphylococcus aureus*, while both the root and bark extracts had a greater effect on *Shigella sonnei*, and the least inhibited bacteria by all extracts was *Serratia marcescens*. The root extract exhibited the highest antibacterial activity and the bark extract demonstrated the least activity. The root and leaf extract inhibited bacterial growth of all test bacteria at the lowest extract concentration tested (0.625 mg/mL) while at the lowest extract concentration the bark extract only inhibited the growth of *S. marcescens* and *S.aureus.* Tables 8-10 summarize the antibacterial activity results expressed as zones of inhibition in mm.

**Table 8**: *Albizia anthelmintica* ethanolic leaf extract bacterial growth inhibition zones at varying extract concentrations

| **Extract (mg/mL)** | **Inhibition zone (mm)** | | |
|---|---|---|---|
| | ***S. marcescens*** | ***S. sonnei*** | ***S. aureus*** |
| **0.625** | 2.04 ± 0.41 | 2.50 ± 0.24 | 2.67 ± 0.47 |
| **1.25** | 2.42 ± 0.12 | 3.83 ± 2.12 | 3.50 ± 0.24 |
| **2.50** | 2.79 ± 0.06 | 3.83 ± 2.12 | 4.50 ± 1.18 |
| **5.00** | 3.88 ± 0.18 | 4.50 ± 1.18 | 4.67 ± 1.41 |
| **10.00** | 4.33 ± 0.47 | 5.92 ± 2.47 | 5.67 ± 1.41 |

**Table 9**: *Albizia anthelmintica* ethanolic root extract bacterial growth inhibition zones at varying extract concentrations

| **Extract (mg/mL)** | **Inhibition zones (mm)** | | |
|---|---|---|---|
| | ***S. marcescens*** | ***S. sonnei*** | ***S. aureus*** |
| **0.625** | 2.78 ± 0.38 | 3.83 ± 0.29 | 4.78 ± 1.68 |
| **1.25** | 2.54 ± 0.98 | 4.56 ± 1.39 | 5.67 ± 1.89 |
| **2.50** | 4.06 ± 0.42 | 5.72 ± 1.46 | 5.22 ± 1.84 |
| **5.00** | 4.33 ± 0.58 | 5.89 ± 1.46 | 5.78 ± 1.84 |
| **10.00** | 4.89 ± 1.02 | 5.28 ± 1.59 | 5.00 ± 0.33 |

**Table 10**: *Albizia anthelmintica* ethanolic bark extract bacterial growth inhibition zones at varying extract concentrations

| **Extract (mg/mL)** | **Inhibition zones (mm)** | | |
|---|---|---|---|
| | ***S. marcescens*** | ***S. sonnei*** | ***S. aureus*** |
| **1.25** | 2.50 ± 0.71 | 3.00 ± 1.41 | 3.50 ± 0.71 |
| **0.625** | 1.50 ± 2.12 | 0.00 ± 0.00 | 3.00 ± 0.00 |
| **2.50** | 3.00 ± 1.41 | 4.50 ± 0.71 | 3.83 ± 1.18 |
| **5.00** | 3.00 ± 1.41 | 5.00 ± 0.71 | 4.33 ± 0.47 |
| **10.00** | 3.50 ± 0.71 | 5.00 ± 0.71 | 5.17 ± 0.24 |

### 4.5.3 Minimum inhibitory concentration

The lowest extract concentration that prevents visible bacterial growth; was determined for all three extracts. The MIC values obtained for the leaf, root and bark extracts of *Albizia anthelmintica* against the test organisms varied from one plant extract to the other, and the results are summarized in table 12. The root extract exhibited the least MIC value (2.50 mg/mL) which was demonstrated against *S. sonnei* and *S. aureus.* A least MIC value demonstrated by the root extract compared to the bark and leaf extracts could be an indication of better antibacterial activity of this extract in comparison to the leaf and bark extracts.

**Table 11**: MIC values for leaves, roots and stem bark ethanolic extracts of *Albizia anthelmintica.*

| **Extract** | **Minimum inhibitory concentration (mg/mL)** | | |
|---|---|---|---|
| | ***S. sonnei*** | ***S. aureus*** | ***S. marcescens*** |
| Bark | 5.00 | 10.00 | 10.00 |
| Leaf | 5.00 | 5.00 | 10.00 |
| Root | 2.50 | 2.50 | 5.00 |

## CHAPTER 5

## DISCUSSION

The phytochemical analysis of leaf, root and stem bark ethanolic extracts of *Albizia anthelmintica* revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins and diterpenes. The detected compounds have been vastly reported for their antimicrobial and antioxidant activities (8, 109). Thus, the presence of these bioactive components in *A, anthelmintica* leaf, root and stem bark ethanolic extracts may account for the antibacterial and antioxidant activity demonstrated by the extracts. The relative amounts of phenolic components in leaves, roots and stem bark extracts were observed to be higher than that of flavonoid components, with the root extract exhibiting the greatest amounts of both phenolic and flavonoid components. Minimal literature was available on the phytochemical investigation of *A. anthelmintica*, with exceptions of phenols isolated from *A. anthelmintica* Egyptian flora (22). In a study by Aliyu *et al (110)*, phytochemical screening of *Albizia chelvalieri* leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, and terpenes while anthraquinones and glycosides were not detected. The present study detected the same compounds; anthraquinones and glycosides where however not detected.

All extracts exhibited a significant ($p<0.05$) concentration depended antibacterial activity against pathogenic strains of *Serratia marcescens* (ATCC 8100), *Shigella sonnei* (ATCC 25931), and *Staphylococcus aureus* (ATCC 25923). The greater antibacterial activity and the lowest MIC value demonstrated by the root extract could be attributed to the high total flavonoid content of this extract, which is in accordance with literature reports that flavonoids possess very good antimicrobial activity (111). Moreover, the antibacterial activity demonstrated by the leaf, root

and stem bark extracts of *Albizia anthelmintica* against the selected test organisms provide scientific evidence for the anecdotal traditional success of this plant in the treatment of various microbial ailments such as diarrhoea, coughs, tuberculosis, malaria and fevers (42, 44). In addition, all extracts showed antibacterial activity against both gram positive (*S. aureus*) and gram negative (*S. marcescens* and *S. sonnei*) bacterial species, which is an indication of a broad spectrum of activity. However, the extracts were not active against the tested *Enterococci* species (*E. faecalis and A. faecalis).* However, a literature search has provided minimal information on the antibacterial investigation of plants belonging to the genus *Albizia,* thus encouraging extensive antibacterial research to be conducted on these plants.

Leaf, roots and stem bark extracts all displayed appreciable DPPH free radical scavenging activity. However, the scavenging activity was lower than that of ascorbic acid. Results obtained in the present study are a good indication that the extracts possess proton-donating ability, and could serve as free radial inhibitors or scavengers. In a study by Tahia *et al* (22), eight phenolic compounds isolated from the leaves of *Albizia anthelmintica* demonstrated remarkable DPPH free radical scavenging activity. The results of the present study are in agreement with the findings by Tahia *et al*. In the present study, all three extracts of *Albizia anthelmintica* demonstrated ferric reducing antioxidant power. The root extract showed relatively higher ferric reducing power compared to the leaf and bark extract, which correlates with the TFC and TPC of the root extract. This signifies that leaves, roots and stem bark ethanolic extracts of *Albizia anthelmintica* are capable of donating electrons that can react with free radicals to convert them into more stable products and thus inhibiting radical chain reactions. The total antioxidant capacity of all the extracts increased significantly with an increase in extract concentration. A relatively high total antioxidant capacity was observed for the root extract, which could be

accounted to the TPC and TFC of this extract in comparison to the leaf and stem bark extract. Commercial antioxidants derived from plant sources are limited, with examples of curcumin, a phenolic compound and major component of *Curcuma longa* and resveratrol, a polyphenolic compound found in the skin of grapes (112). Thus, the findings of the study indicates the potential of the leaf, root and stem bark extracts of *Albizia anthelmintica* as sources of antioxidant compounds.

# CHAPTER 6

## CONCLUSION

The study clearly revealed that the leaf, root and stem bark ethanolic extracts of *Albizia anthelmintica* contains compounds with antibacterial and antioxidant properties and suggests that the plant could be a source of potential antibacterial and antioxidant agents. The observed appreciable antibacterial and antioxidant activity of the root extract might be linked to the high phenolic and flavonoid contents of this extract compared to the leaf and stem bark extract. In addition, the findings from this study support the traditional uses of these three plant parts. Moreover, the findings of this study add value to the traditional uses of *A. anthelmintica*. Locally, *A. anthelmintica* grows in the wild thus conservation of this plant is necessary due to the significant in vitro antibacterial and antioxidant activities demonstrated by *A. anthelmintica* in this study.

# CHAPTER 7

## RECOMMENDATIONS

An in-depth phytochemical investigation of the leaf, root and stem bark extracts of *Albizia anthelmintica i*s highly recommended. This is necessary in order to isolate and characterize compounds with antioxidant and antibacterial activity in *A. anthelmintica* extracts. This will enable the establishment of structure-activity relationships, which is an important aspect in the discovery of new drugs. Further investigations could include screening the extracts for antiviral and antifungal activity, in order to determine whether the extracts have activity against microorganisms other than bacteria. *In vivo* and toxicological studies of *A. anthelmintica* extracts are further recommended.

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## APPENDIX 1

UNAM
UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

**Ethical Clearance Reference Number: FOS/97/2016** **Date: 29 April, 2016**

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

**Title of Project:** ANTIBACTERIAL, ANTIOXIDANT AND PHYTOCHEMICAL INVESTIGATION OF ALBIZIA ANTUNESIANA AND ALBIZIA ATHELMINTICA LEAVES, ROOTS AND BARK

**Nature/Level of Project**: Masters

**Researcher**: Teopolina N. Nawinda

**Student Number** : 201148757

**Faculty**: Faculty of Science

**Supervisor** : Dr. P. Kapewangolo

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:
  (i). withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
  (ii). request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

PP

Dr. H. Kapenda
Director –Centre for Research and Publications
ON BEHALF OF UREC

MINISTRY OF ENVIRONMENT AND TOURISM

# RESEARCH/COLLECTING PERMIT

**Permit Number 1992/2014**
**Valid from 1 January 2015 to 31 December 2015**

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: **Dr. P. Kapewangolo**
Address: **Department of Chemistry and Biochemestry**
**Faculty of Science**
**Universtity of Namibia**
**Windhoek**
Coworkers: **M. Kandawa- Schulz and M. Hedimbi**

Biological screening of medicinal plants / herbs used traditionally for antiviral treatment at the University of Namibia, subject to attached conditions.

**IMPORTANT:** This permit is not valid if altered in any way.

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
2014 -12- 12
WINDHOEK
Private Bag 13306, Windhoek
Tel: 2842111 • Fax: 258861

Authorizing Officer

**IMPORTANT**
This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Warden, email clouw@met.na
Private Bag 13306, Windhoek, Namibia

NAMIBIAN POLICE FORCE
KHOMAS REGION
14 JAN 2016
KATUTURA

## RESEARCH/COLLECTING PERMIT CONDITIONS

********

1. The permission of the land owner is required to work/collect on private lands.
2. The permission of the concession holder is required to work/collect in concession areas.
3. The permission of the communal authority is required to work/collect in communal areas.
4. Duplicates of publications and / or final report should be made available to MET Resource Centre and also the final report is to be submitted to the NBRI reference library.
5. Duplicate voucher specimens of plants collected are to be lodged with National Herbarium at NBRI.
6. A material transfer agreement (MTA) must be signed with the NBRI for any plant material and / or extracts or derivatives thereof to be exported prior to the export thereof.
7. The specimens and their derivatives may be used for the purposes of this study only and may not be patented, commercialised, donated or sold to a third party without the written consent of the Ministry of Environment and Tourism.
8. All results (raw materials) or technology derived directly or indirectly from this research must be made available free of charge without reservations to the Ministry of Environment and Tourism.
9. Please submit a report on the work conducted under this permit to this office not later than one month after the expiry of this permit as well as to regional office where you have reported.
10. Applications for renewal of this permit must reach this office at least three months prior to the expiry of this permit.
11. Habitat destructive collecting methods must not to be used.
12. Local Veterinary export regulations will apply.
13. For export of CITES species samples you need CITES import permit from the destination so that you can be issued a CITES permit.
14. All field teams must be in possession of the permit (or copy) and permits must accompany the transport of specimens.
15. You are subject to all conditions listed on the entry permit to any of the protected areas, unless specifically exempted.
16. It is your responsibility to make the necessary contacts and arrangements as specified above.

Ministry of Agriculture, Water and Forestry

**National Herbarium of Namibia (WIND)**

## Identification Report

**Report No.: 2016/374**

**25 January 2016**

**Collector/s:** Mrs T.ND. Nawinda
**Address:** University of Namibia
Windhoek

| Number | ID cat. | Identification |
|---|---|---|
| TN 02 | 1 | Albizia anthelmintica (A. Rich.) Brongn. |
| TN 01 | 1 | Albizia anthelmintica (A. Rich.) Brongn. |

Comment:

**Curator**
**National Herbarium of Namibia (WIND)**

**Identification categories: 1. Certain identification 2. Closest to 3. Certain to genus only 4. Unable to identify**

Private Bag 13184, Windhoek Tel: +264 - 61 - 202 - 2021 Fax: +264 - 61 - 259 - 153 e-mail: essiek@nbri.org.na

**APPENDIX 2**

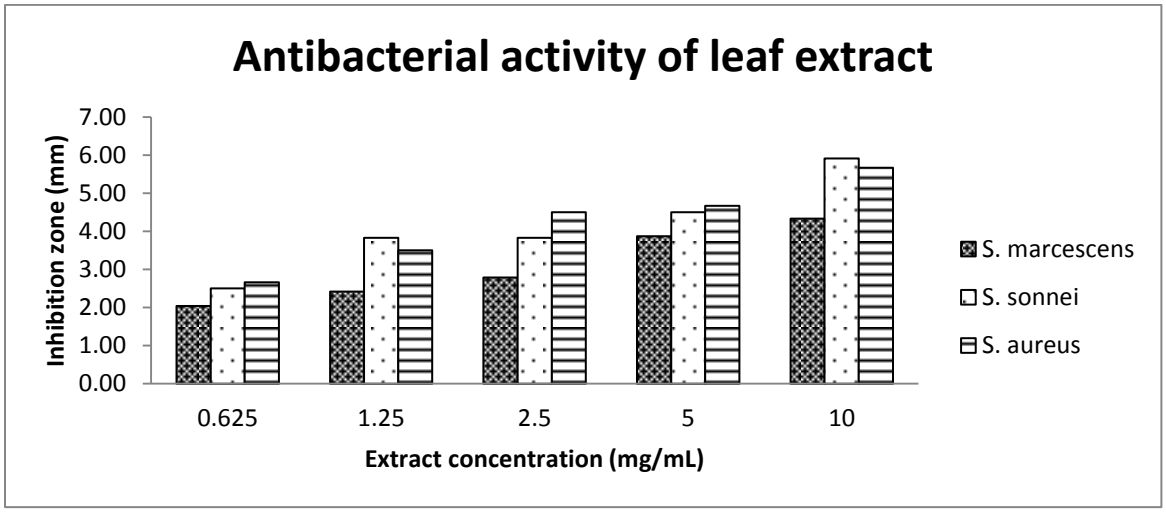


**Figure 6**: Bacterial growth inhibition by *A. anthelmintica* leaf ethanolic extract at varying concentrations against *S. marcescens, S. sonnei and S. aureus.*

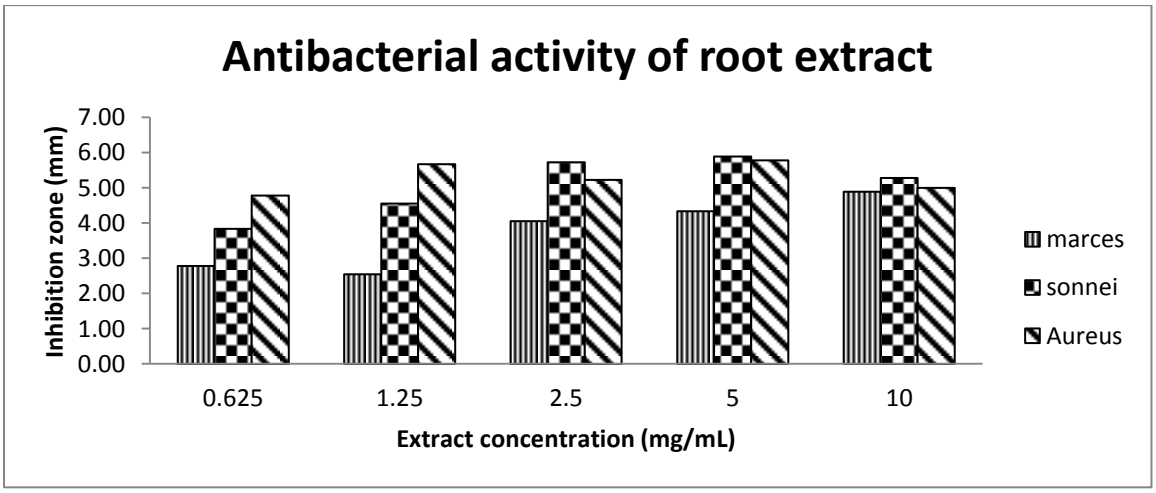


**Figure 7**: Bacterial growth inhibition by *A. anthelmintica* root ethanolic extract at varying concentrations against *S. marcescens, S. sonnei and S. aureus.*

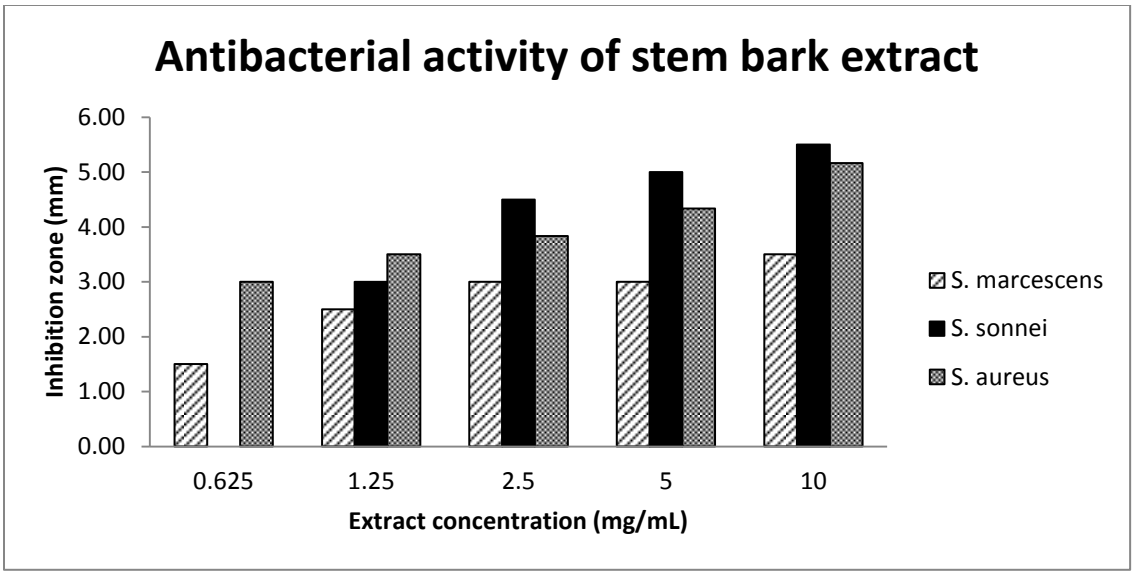


**Figure 8**: Bacterial growth inhibition by *A. anthelmintica* stem bark ethanolic extract at varying concentrations against *S.marcescens, S. sonnei, and S. aureus.*

# APPENDIX 3

**Figure 9**: *Albizia anthelmintica* stem

**Figure 10**: *Albizia anthelmintica* fresh leaves

**Figure 11**: *Albizia anthelmintica* dry stem bark

**Figure 12**: *Albizia anthelmintica* crushed stem bark

**Figure 13**: *Albizia anthelmintica* dry roots

**Figure 14**: *Albizia anthelmintica* crushed roots

**Figure 15**: *Albizia anthelmintica* crushed leaves